

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Bennett *et al.*

Appl. No. 10/622,088

Filed: July 18, 2003

For: **Viral Vectors Containing
Recombination Sites**

Confirmation No.: 1853

Art Unit: 1648

Examiner: Horning, Michelle S.

Atty. Docket: IVGN 332

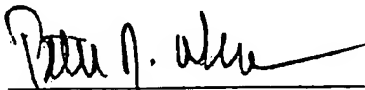
Declaration Under 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Peter J. Welch, a resident of 4689 E. Talmadge Drive, San Diego, California declare as follows:

1. I am an inventor on the present application, together with Robert Bennett, Kenneth E. Franke, Kenneth Frimpong, Steven Harwood, and Knut Madden.
2. The invention claimed in the above identified Patent Application was conceived and reduced to practice prior to October 31, 2001, the publication date of the cited reference, Loftus *et al.*
3. Attached as Exhibit A are notebook records relating to the conception and reduction to practice of the invention. Notebook 4, pages 37-39, dated August 16 and August 17, 2001, describe the construction of the pLenti6/V5-DEST vector shown in Figure 36A of the above referenced application. The vector was constructed by ligation of an Eco RV Dest cassette into pRRL6V53stops followed by transformation into DB 3.1 cells. A similar reaction is described in the Notebook page dated October 3, 2001.
4. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of

the United States Code, and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.



Peter J. Welch

July 23, 2008
Date.

ligation of EcoRV Dst cassette A into pRRL6V53 stops and pRRL6V53 stops ppt digested with EcoRV and CIP-treated.

	1	2	3	4
10x ligation buffer	1	1.1	1.1	1.1
pRRL6V53 stops ppt	3	3	3	3
Dst cassette A	0	3	4	5
dH ₂ O	5	3	2	1
Ligase	1	1	1	1
	10ul	~11ul	~11ul	~11ul

These ligation reactions were incubated @ RT for 1 hr. 3ul was used to transform DB3.1 cells using the std. protocol

opt)

	5	6	7	8
10x ligation buffer	1	1.1	1.1	1.1
pRRL6V53 stops ppt	3	3	3	3
Dst Cassette A	0	3	4	5
dH ₂ O	5	3	2	1
Ligase	1	1	1	1
	10ul	~11ul	~11ul	~11ul

30min on ice, heat-shocked @ 42 for 45 sec. on ice for 2'. Added 0.9ml SOC. Incubate @ 37°C for 1h prior to plating on LB can plates. Used pCDNA4/1K03/1 as control. 200ul of each transformation was plated.

After seeing that the Nant digest of pCDNA3.1/VS45A looked complete, I formulated a larger digest in order to generate lentivectors with SV40-Neo.

10x NruI buffer	7.0
pCDNA3.1/VS45A	5.0
dH ₂ O	53.0
NruI	5.0
	70ul

This reaction was incubated @ 37°C for overnight.

Continued on Page

Read and Understood By

radley D Howard
Signed

8/16/07
Date

U Henry
Signed

8/17/07
Date

8/17/01 - Results of Best Vector transformations/constructions.

Transformations# colonies

1 DB3.1 only	0
2 pCDNA4/ires/DEST	TNTC
3 lig 1	0
4 lig 2	412
5 lig 3	514 → Bev will grow up 20 colonies
6 lig 4	1122
7 lig 5	0
8 lig 6	680
9 lig 7	726 → Bev will grow up 20 colonies
10 lig 8	765

Bev was kind enough to carry some aspects of the lentiviral project forward while I am gone on vacation.

I gave her some DVAs and some general guidelines as described on the next page.

Continued on Page 39

Read and Understood By

Bradley D Howard
Signed

8/17/01
Date

Harry Y
Signed

8/17/01
Date

Hi Ben,

Here goes!

Grow up up #6 and #15 from the upDNAs/cultures that you analysed for me.

These plasmids should be called pRL6FPVS3stoppt #6 and #15. Make Endofire preps using the Qiagen kit and protocol. Grow up 2x 100ml cultures each.

As for the pRL6FPVS3stop, the DNAs are name pRL6FPVS3stop 8-2 and pRL6FPVS3stop 8-4. Use 8-2 since the conc of DNA is higher. You will need to transform HB101 only, no other strain will work. Grow up ~~up~~ ^{the} colonies from what results from the transformation. Isolate them separately. These DNAs need to be grown up using the Endofire kit (Qiagen). Remember to grow up 2x 100ml cultures (TB + amp 50mg/ml).

DEST Vectors

Take ~20 colonies from plate 3, ~~on~~ these would correspond to the DEST version pRL6VS3stopptDEST. Take 20 colonies from plate 7. These colonies would correspond to the DEST version pRLVS3stopptDEST.

Continued on Page 40

Read and Understood By

Reading D. Howard

Signed

8/17/01

Date

Harry G

Signed

8/17/01

Date

Continued From Page

10/1/01 SHORILL'S HAVING SOME TROUBLE GETTING TEST CASSETTE WORKING
SO I'LL TRY IN PARALLEL:

p_{RL6}/VS & $p_{RL6}/VS/ppt$ $5_{\mu s}$ DNA from BEV

	10 μ l		40 μ l
H ₂ O	33 μ l		3 μ l
MDX #3	5 μ l	\rightarrow 1.5 μ l	5 μ l \rightarrow 1.5 μ l
EORV	2 μ l		2 μ l
	<u>50 (100% μl)</u>		<u>50</u>

37°C x 2hrs

→ 1.5 μ

CLEAN UP, ELITE SQUAD

USE WELIGATION WITH REST CASSETTE B

	VS -	VS +	ppt -	ppt +
PRRLUG VS	2.0	2.0	—	—
PRRLUG VS ppt	—	—	1.0	1.0
H ₂ O	4.06	4.0	5.07	5.0
10X UG BUC	1.0	1.0	1.0	1.0
UGASE	1.0	1.0	1.0	1.0
NET CUS. B 10 ¹¹ / µl	2.0 —	2.0	2.0 —	2.0

-10-

16°C O/N

02/01 Then OB3.1 100 μ D AND RATE: 100 μ E EACH \rightarrow LB-amp
 Par 46 100 μ D " \rightarrow LB-amp + Cam
 REST " \rightarrow LB-amp + Cam

10/3/01 EXERCISE 1: $V5-$, ppt- = NO COLONIES
 $V5+$, ppt+ = LOTS OF COLONIES } ON Amp + Gm PLATED

YEP!

SHARUKH WILL TAKE IT FROM HERE...

Continued on Page

Read and Understood By

Signed John A. We Date 3/21/01

Signed John G. Ayres Date 11/7/02